

onto 4 mg alum and sacrificed after 15 days. Spleens were excised and homogenized in a tissue grinder, washed twice, and maintained in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 0.0005% 2-mercaptoethanol. Spleen cell cultures were established (2-3 million cells/ml, 0.2 ml/well in quadruplicate, 96-well plates) in the presence or absence of DNP-KLH (10 ng/ml). Test compounds (2 µg/ml and 50 ng/ml) were added to the spleen cell cultures containing antigen and incubated at 37° C for 8 days in an atmosphere of 10% CO₂.

[0032] Culture supernatants were collected after 8 days and Ig's were measured by a modification of the specific isotype-selective ELISA assay described by Marcelletti and Katz (Supra). The assay was modified to facilitate high throughput. ELISA plates were prepared by coating with DNP-KLH overnight. After blocking with bovine serum albumin (BSA), an aliquot of each culture supernatant was diluted (1:4 in phosphate buffered saline (PBS) with BSA, sodium azide and Tween 20), added to the ELISA plates, and incubated overnight in a humidified box at 4° C. IgE levels were quantitated following successive incubations with biotinylated-goat antimouse IgE (b-GAME), AP-streptavidin and substrate.

[0033] Antigen-specific IgG1 was measured similarly, except that culture supernatants were diluted 200-fold and biotinylated-goat antimouse IgG1 (b-GAMG1) was substituted for b-GAME. IgG2a was measured in ELISA plates that were coated with DNP-KLH following a 1:20 dilution of culture supernatants and incubation with biotinylated-goat antimouse IgG2a (b-GAMG2a). Quantitation of each isotype was determined by comparison to a standard curve. The level of detectability of all antibody was about 200-400 pg/ml and there was less than 0.001% cross-reactivity with any other Ig isotype in the ELISA for IgE.

In Vivo Assay

[0034] Compounds found to be active in the ex vivo assay (above) were further tested for their activity in suppressing IgE responses in vivo. Mice receiving low-dose radiation prior to immunization with a carrier exhibited an enhanced IgE response to sensitization with antigen 7 days later. Administration of the test compounds immediately prior to and after antigen sensitization, measured the ability of that drug to suppress the IgE response. The levels of IgE, IgG1 and IgG2a in serum were compared.

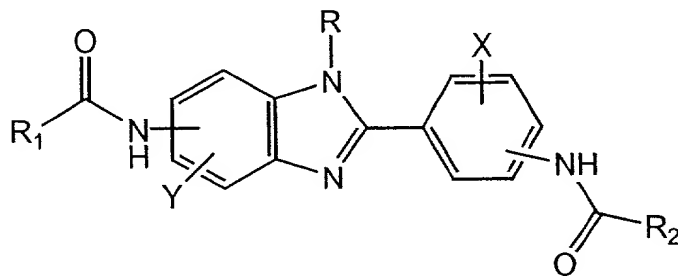
[0035] Female BALB/cByj mice were irradiated with 250 rads 7 hours after initiation of the daily light cycle. Two hours later, the mice were immunized i.p. with 2 μ g of KLH in 4 mg alum. Two to seven consecutive days of drug injections were initiated 6 days later on either a once or twice daily basis. Typically, i.p. injections and oral gavages were administered as suspensions (150 μ l/injection) in saline with 10% ethanol and 0.25% methylcellulose. Each treatment group was composed of 5-6 mice. On the second day of drug administration, 2 μ g of DNP-KLH was administered i.p. in 4 mg alum, immediately following the morning injection of drug. Mice were bled 7-21 days following DNP-KLH challenge.

[0036] Antigen-specific IgE, IgG1 and IgG2a antibodies were measured by ELISA. Periorbital bleeds were centrifuged at 14,000 rpm for 10 min, the supernatants were diluted 5-fold in saline, and centrifuged again. Antibody concentrations of each bleed were determined by ELISA of four dilutions (in triplicate) and compared to a standard curve: anti-DNP IgE (1:100 to 1:800), anti-DNP IgG2a (1:100 to 1:800), and anti-DNP IgG1 (1:1600 to 1:12800).

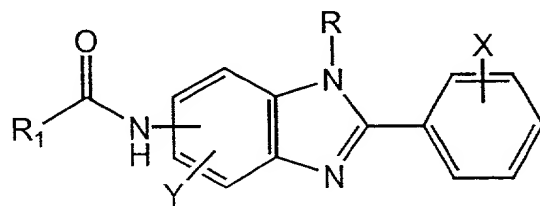
Benzimidazole Inhibitors of IgE

[0037] Several species embraced by the following generic formula were synthesized and evaluated for their effectiveness in down-regulating IgE in the *ex vivo* and *in vivo* assays.

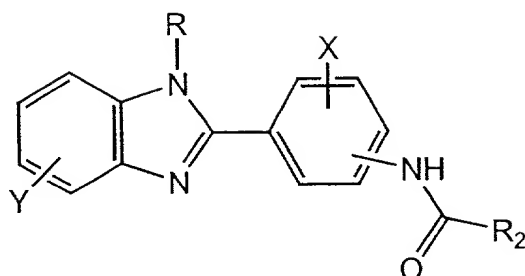
Genus A,



Genus B, and



Genus C,



[0038] wherein X and Y are independently selected from the group consisting of H, alkyl, alkoxy, aryl, substituted aryl, hydroxy, halogen, amino, alkylamino, nitro, cyano, CF₃, OCF₃, CONH₂, CONHR and NHCOR₁;

[0039] wherein R is selected from the group consisting of H, CH₃, C₂H₅, C₃H₇, C₄H₉, CH₂Ph, CH₂C₆H₄-F(p-), COCH₃, CO₂CH₂CH₃, aminoalkyl and dialkylaminoalkyl; and

[0040] wherein R₁ and R₂ are independently selected from the group consisting of H, aryl, heteroaryl, thiophene, pyridyl, thiazolyl, isoxazolyl, oxazolyl, pyrimidinyl, substituted aryl, substituted heteroaryl, substituted thiophene, substituted pyridyl, substituted thiazolyl, substituted isoxazolyl, substituted oxazolyl, cycloaryl, cycloheteroaryl, quinolinyl, isoquinolinyl, substituted cycloaryl, substituted cycloheteroaryl, substituted quinolinyl, substituted isoquinolinyl, multi-ring cycloaryl, multi-ring cycloheteroaryl, benzyl, heteroaryl-methyl, substituted benzyl, substituted heteroaryl-methyl alkyl, dialkylaminoalkyl, cycloalkyl, cycloalkyl containing 1-3 heteroatoms, substituted cycloalkyl, substituted cycloalkyl containing 1-3 heteroatoms, multi-ring cycloalkyl, multi-ring cycloalkyl containing 1-3 heteroatoms, fused-ring aliphatic, fused-ring aliphatic containing 1-3 heteroatoms, cyclopropyl, substituted cyclopropyl, cyclobutyl, substituted cyclobutyl, cyclopentyl, pyrrole, piperidine, substituted cyclopentyl, cyclohexyl, substituted cyclohexyl, cycloheptyl, substituted cycloheptyl, bicycloheptyl, substituted pyrrole, substituted piperidine, bicyclooctyl, bicyclononyl, substituted bicycloalkenyl, adamantyl, substituted adamantyl and the like, wherein at least one of R₁ and R₂ are aromatic groups or heteroaromatic groups.

[0041] The substituents on said substituted aryl, substituted heteroaryl, substituted thiophene, substituted pyridyl, substituted thiazolyl, substituted isoxazolyl, substituted oxazolyl, substituted cycloaryl, substituted cycloheteroaryl, substituted quinolinyl,